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A Limited Set of SH2 Domains Binds BCR through a High-Affinity Phosphotyrosine-Independent Interaction

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SH2 (*src* homology region 2) domains are implicated in protein-protein interactions involved in signal transduction pathways. Isolated SH2 domains bind proteins that are tyrosine phosphorylated. A novel, phosphotyrosine-independent binding interaction between BCR, the Philadelphia chromosome breakpoint cluster region gene product, and the SH2 domain of its translocation partner c-ABL has recently been reported. We have examined the ability of additional SH2 domains to bind phosphotyrosine-free BCR and compared this with their ability to bind tyrosine-phosphorylated c-ABL 1b. Of 11 individual SH2 domains examined, 8 exhibited relatively high affinity for c-ABL 1b, whereas only 4 exhibited relatively high affinity for BCR. Binding of tyrosine-phosphorylated c-ABL 1b by the relatively high-affinity ABL and ARG SH2 domains was quantitatively analyzed, and equilibrium dissociation constants for both interactions were estimated to be in the range of 5×10^{-7} M. The ABL SH2 domain exhibited relatively high affinity for phosphotyrosine-free BCR as well; however, this interaction appears to be about two orders of magnitude weaker than binding of tyrosine-phosphorylated c-ABL 1b. The ARG SH2 domain exhibited relatively weak affinity for BCR and was determined to bind about 10-fold less strongly than the ABL SH2 domain. The ABL and ARG SH2 domains differ by only 10 of 91 amino acids, and the substitution of ABL-specific amino acids into either the amino- or carboxy-terminal half of the ARG SH2 domain was found to increase its affinity for BCR. We discuss these results in terms of a model which has been proposed for peptide binding by class I histocompatibility glycoproteins.

The transduction of signals from the cell surface to the nucleus is a fundamental process in the regulation of cell growth and differentiation (5, 13). SH2 (*src* homology region 2) domains have been implicated in effecting specific interactions between components of signal transduction pathways. The SH2 domain was initially identified as a homologous region of approximately 100 amino acids immediately upstream of the catalytic domains of both the *src* and *fps* nonreceptor protein tyrosine kinases that was important for $p130^{gag\text{-}fps}$ tyrosine kinase activity in rat-2 fibroblasts (36). Subsequently, SH2 domains have been identified in several proteins that are not tyrosine kinases (15). SH2 domains mediate binding to phosphotyrosine-containing proteins and have been demonstrated to direct interactions with activated growth factor receptors as well as with other tyrosine-phosphorylated components of signal transduction pathways (15, 19).

Binding specificity exhibited by isolated SH2 domains in vitro may serve as a potential indicator of physiological interactions in vivo. Phospholipase Cγ (PLCγ) is complexed with and tyrosine phosphorylated by the activated platelet-derived growth factor and epidermal growth factor receptors (18, 20, 22, 28, 41, 42) but not by the activated colony-stimulating factor 1 receptor (7). Similar observations have been reported for GTPase-activating protein (GAP) (8, 14, 29, 35). The SH2 domains of both PLCγ and GAP bind the tyrosine-phosphorylated epidermal growth factor and platelet-derived growth factor receptors in vitro but do not bind the tyrosine-phosphorylated colony-stimulating factor 1 receptor (1, 21, 27, 30).

The isolated SH2 domain of ABL has recently been demonstrated to bind the BCR gene product in vitro through a novel, phosphotyrosine-independent interaction (33). This binding interaction is dependent on BCR phosphorylation and probably requires either phosphoserine or phosphothreonine. A region within the first exon of BCR which contains two stretches of high serine content is required for ABL SH2 binding. Baculovirus-expressed BCR and c-ABL 1b have been shown to coimmunoprecipitate with either BCR-specific or ABL-specific antibodies, suggesting that the binding interaction between the ABL SH2 domain and BCR can take place in the context of the full-length molecules (33). In contrast, baculovirus-produced GAP, which contains two SH2 domains, did not detectably coimmunoprecipitate with BCR (33). Specific binding interactions between BCR and SH2-containing proteins might reflect inherent differences in BCR-binding capability among SH2 domains or might be determined by the sequence context in which SH2 domains are located.

In this study, we examined a panel of 11 SH2 domains from eight different proteins for in vitro binding of serine/threonine-phosphorylated BCR. Binding of tyrosine-phosphorylated c-ABL 1b by the same panel of SH2 domains was analyzed for comparison. Only a subset of the SH2 domains that bound c-ABL 1b with high affinity also exhibited high-affinity binding for BCR. The ability of SH2 domains to mediate selective binding interactions through phosphoserine/phosphothreonine may be important for directing spe-

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cific protein-protein interactions involved in signal transduction pathways.

MATERIALS AND METHODS

Preparation of GST fusion proteins. GST fusions with ARG SH2, GAP(C) SH2, and LCK SH2 were constructed by amplification of these regions from human leukemia cell line K562 cDNA by poly-merase chain reaction (PCR) and subcloning into pGEX-3X (Pharmacia). The GST fusion with FPS SH2 was constructed by PCR amplification of this region from subcloned $p130^{gag\text{-}fps}$-encoding cDNA. The other fusion proteins have been described previously: Src SH2 (16); GAP(N) SH2, GAP SH2/3/2, PLCγ(N) SH2, PLCγ(C) SH2, and PLCγ(N+C) (1); ABL SH2 (33); and P85(N) SH2 and P85(C) SH2 (27). PLCγ(C) SH2 was previously reported not to bind well to activated growth factor receptors (1); however, this was subsequently determined to be because residues that are important for binding were missing from the C terminus. Seven additional C-terminal residues are present in the form of PLCγ(C) SH2 used in this study. The GST fusion proteins used in this study contained the following amino acids: ABL SH2, human c-ABL 1b amino acids 139 to 238; ARG SH2, human ARG amino acids 164 to 270; PLCγ(N) SH2, bovine PLCγ1 amino acids 547 to 659; PLCγ(C) SH2, bovine PLCγ1 amino acids 663 to 759; PLCγ(N+C) SH2, bovine PLCγ1 amino acids 547 to 759; GAP(N) SH2, human ras-GAP amino acids 178 to 277; GAP(C) SH2, human ras-GAP amino acids 346 to 447; GAP SH2/3/2, human ras-GAP amino acids 171 to 448; P85(N) SH2, bovine p85α phosphatidylinositol 3-kinase (PI3 kinase) subunit amino acids 312 to 444; P85(C) SH2, bovine p85α PI3 kinase subunit amino acids 612 to 722; SRC SH2, $p60^{v\text{-}src}$ amino acids 148 to 251; FPS SH2, $p130^{gag\text{-}fps}$ amino acids 806 to 914; and LCK SH2, human c-LCK amino acids 122 to 230.

The ABL/ARG and ARG/ABL composite SH2 domains were constructed by utilizing a unique *Pst*I site which is in the SH2 domain of ARG but not in the SH2 domain of ABL. This site overlaps amino acids T-53 and A-54 of the ARG SH2 domain, which are not in the ABL SH2 domain (see Fig. 4). Amino and carboxy portions of the ABL SH2 domain were amplified by PCR with ARG-specific *Pst*I-coding sequences substituted at the end. These PCR-generated fragments were then joined at the *Pst*I site with ARG SH2-coding sequences as GST fusion constructs. ABL/ARG(18L) was constructed by first altering sequences coding for Gly at position 18 in the ABL SH2 domain to code for Leu (Fig. 4) by using the Amersham oligonucleotide-directed in vitro mutagenesis system as described by the manufacturer. This mutation was confirmed by dideoxy sequence analysis. A PCR fragment with the ARG-specific *Pst*I site added was then generated from this mutated ABL SH2 domain and was joined with ARG SH2-coding sequences to produce the ABL/ARG(18L) GST fusion construct. ARG (9N) and ARG(35R) were constructed by using a unique *Esp*I site located in the coding sequences for amino acids 14 to 16 of both the ABL and ARG SH2 domains. This site differs between the ABL and ARG SH2 domains in a central, degenerate base pair. To construct ARG(9N), the ARG SH2 domain clone was used as a PCR template to generate a fragment with an ABL-specific *Esp*I site. This fragment was joined at the *Esp*I site with ABL SH2-coding sequences as a GST fusion construct. The expected ligation products were verified by restriction endonuclease digestion and dideoxy sequencing.

Procedures for expressing GST fusion proteins in *Escherichia coli* and for extracting the fusion proteins and coupling them to glutathione-Sepharose 4B (Pharmacia) have been previously described in detail (33).

SH2-binding assay for [^{35}S]methionine-labeled BCR and c-ABL 1b proteins. The generation of cDNAs encoding full-length BCR (23, 39) and c-ABL 1b (31, 32), their incorporation into the baculovirus expression vector pAcC12 (34), and the preparation of recombinant baculoviruses have been described in detail in the references cited. The form of c-ABL 1b used in this study was a murine-human hybrid which differs from human c-ABL 1b by two conservative amino acid changes encoded within the first exon and by an additional codon at the first- and second-exon splice junction (2, 31, 38). Lysates containing ^{35}S-labeled, baculovirus-expressed BCR or c-ABL 1b from infected Sf9 cells were prepared for use in binding assays as previously described (33). Unless otherwise indicated, binding reactions were carried out with approximately 35 μg of each of the GST-SH2 fusion proteins coupled to an equalized packed volume of glutathione-Sepharose 4B beads (Pharmacia) in a total volume of 1.2 ml containing 200 μl of Sf9 lysate and 1.0 ml of incubation buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; pH 7.0], 150 mM NaCl, 0.1% Triton X-100, 10% glycerol, 0.5 mM Na_3VO_4, 0.1 mM Na_2MoO_4, 20 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 25 μg leupeptin per ml). Incubations were carried out on a rocking platform at 4°C for 2 h. The beads were then washed three times with 1 ml of incubation buffer and boiled in 45 μl of 2× sample buffer, of which 20 μl was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. ^{35}S-labeled BCR and c-ABL 1b were visualized by fluorography. Quantitation of intact GST and GST-SH2 fusion proteins coupled to beads was determined by comparison of bands on a Coomassie blue-stained gel with a dilution series of bovine serum albumin (BSA). Details of each experiment and specific modifications of this general procedure are described in the figure legends.

RESULTS

Only a subset of SH2 domains that exhibit relatively high affinity for c-ABL 1b also bind phosphotyrosine-free BCR with relatively high affinity. A panel of bacterially expressed GST-SH2 fusion proteins has been assayed for in vitro binding of full-length c-ABL 1b and BCR proteins. Baculovirus-expressed c-ABL 1b (145 kDa), the protein product of one of two different c-*ABL* transcripts which are generated by differential splicing of alternative first exons (2, 38), was phosphorylated on tyrosine, serine, and threonine residues. Baculovirus-expressed BCR (160 kDa) contained phosphoserine and phosphothreonine but no detectable phosphotyrosine (33). SH2 domains were obtained from members of the *src*, *ABL*, and *fps* families of cytoplasmic tyrosine kinases as well as from the nontyrosine kinases GAP, PLCγ, and the p85 subunit of PI3 kinase (Fig. 1). The relative binding of c-ABL 1b and BCR to equalized amounts of each of the different GST-SH2 fusion proteins (Fig. 2C) was analyzed by polyacrylamide gel electrophoresis and fluorography of the ^{35}S-labeled proteins (Fig. 2A and B).

Only 4 individual SH2 domains of the 11 that were examined bound a relatively large amount of phosphotyrosine-free BCR. These were the ABL SH2 domain, the C-terminal SH2 domain of PLCγ, the N-terminal SH2 domain of GAP, and the SRC SH2 domain (Fig. 2B). In contrast, 8 of the 11 SH2 domains bound relatively large

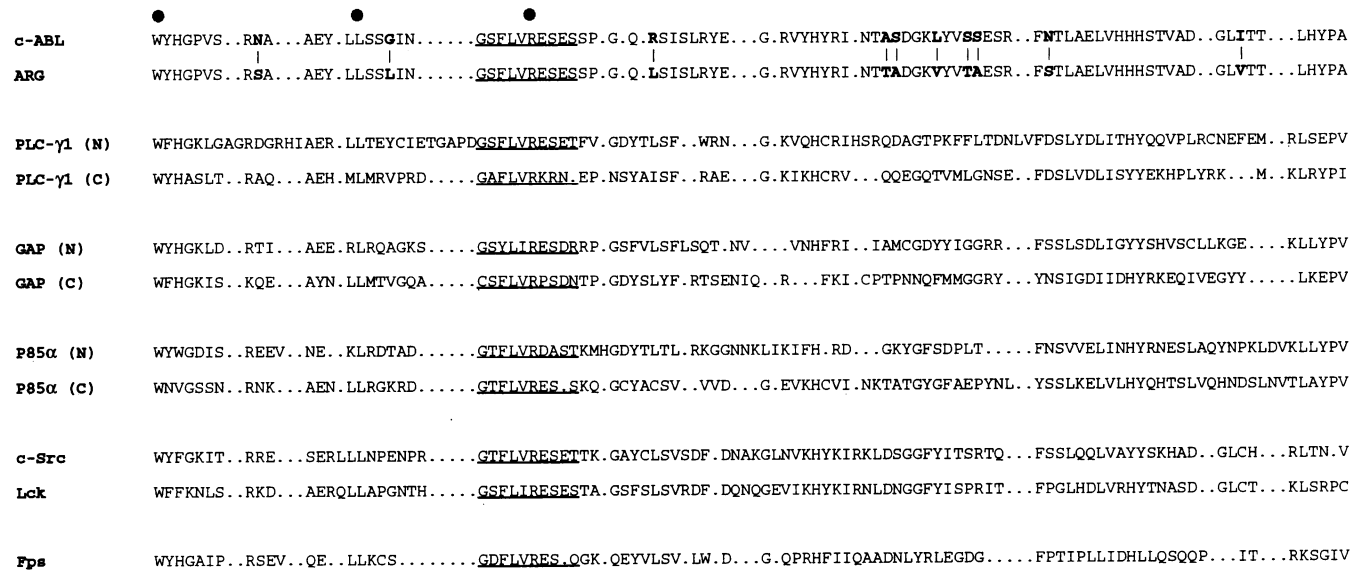

FIG. 1. Amino acid sequence alignment of SH2 domains assayed for BCR binding. Sequences are grouped according to family and aligned as previously reported (15). Three amino acids which are invariant among all SH2 domains are marked with dots at the top (15). Amino acids which differ between the SH2 domains of ABL and ARG are indicated by boldface letters with a line between them. The conserved FLVRES motif in each SH2 domain is underlined. This is based on the consensus sequence GS/TFLVRESES/T, each amino acid of which is present in >50% of recognized SH2 domains (26).

amounts of tyrosine-phosphorylated c-ABL 1b (Fig. 2A). Three SH2 domains bound relatively low levels of both c-ABL 1b and BCR. These were the N-terminal SH2 domain of the p85 subunit of PI3 kinase, the FPS SH2 domain, and the LCK SH2 domain (Fig. 2A and B). The four other SH2 domains that bound relatively small amounts of BCR were the ARG SH2 domain, the N-terminal SH2 domain of PLCγ, the C-terminal SH2 domain of GAP, and the C-terminal SH2 domain of the p85 subunit of PI3 kinase (Fig. 2B).

To quantitatively assess how differences in BCR binding observed in the single-point assay (Fig. 2) relate to actual differences in affinity, constant amounts of baculovirus-expressed c-ABL 1b and BCR were assayed for binding against a dilution series of the ABL and ARG SH2 domains. These two SH2 domains had been observed to bind relatively high and low levels of BCR, respectively. The highest concentration of GST-SH2 fusion protein in each dilution series was comparable to that used in the single point assay. Threefold serial dilutions of GST-SH2 fusion protein were mixed with glutathione-Sepharose 4B beads coupled to GST alone to maintain a constant concentration of total coupled protein and beads in each reaction mixture. Both dilution series were visualized by Coomassie blue staining (Fig. 3C).

The binding profiles of the ABL and ARG SH2 domains obtained with tyrosine-phosphorylated c-ABL 1b are nearly identical (Fig. 3A). In contrast, dilutions of the ARG SH2 domain bind less phosphotyrosine-free BCR than comparable concentrations of ABL SH2 (Fig. 3B). Comparison of lanes 4 and 12 of Fig. 3B indicates that an approximately 10-fold-higher concentration of the ARG SH2 domain relative to the ABL SH2 domain was required in order to bind a comparable amount of BCR. The highest concentrations of the GST-SH2 fusion proteins examined in this assay did not appear to achieve maximal binding of either c-ABL 1b or BCR. Because the single-point binding assays in Fig. 2 were performed under these nonsaturating conditions, the differences observed in c-ABL 1b or BCR binding should reflect actual differences in affinity.

The equilibrium dissociation constant for tyrosine-phosphorylated c-ABL 1b interacting with either the ABL or ARG SH2 domain was determined by Scatchard analysis of the data in Fig. 3A to be in the range of 5×10^{-7} M. The strength of phosphotyrosine-independent SH2 binding of BCR appears to be substantially lower. From the data in Fig. 3B, the equilibrium dissociation constant for BCR binding to the ABL SH2 domain was estimated to be on the order of 10^{-5} M. For the ARG SH2 domain, which exhibited low affinity for BCR, the dissociation constant for BCR binding was determined to be on the order of 10^{-4} M, although this represents a rough estimate because of the low number of counts that were bound.

The actual strength of the different binding interactions may be greater than indicated by the calculated dissociation constants because the calculated values are based on the assumption that all of the ^{35}S-labeled molecules that were present in solution were capable of being bound by the SH2 domains. However, specific phosphorylations are required for SH2 binding, and these phosphorylations are not present on 100% of the molecules. This may also pose a problem for comparisons between the dissociation constants calculated for BCR and c-ABL 1b, since the phosphorylations required for each of these two proteins to bind SH2 domains are different and are probably not present at the same relative frequencies.

The ability of specific SH2 domains to bind BCR with high affinity does not simply correlate with the degree of overall amino acid sequence homology between them. Having demonstrated that only a limited set of SH2 domains exhibit high affinity for BCR, we were interested in identifying determinants in the primary sequence that might account for this binding specificity. The ABL and ARG SH2 domains are the two most highly homologous SH2 domains in this set (Fig. 1), yet they differ by an order of magnitude in their ability to bind BCR. Out of 91 amino acids, 10 are not conserved, which reduces considerably the number of amino acids that might account for the observed binding differential.

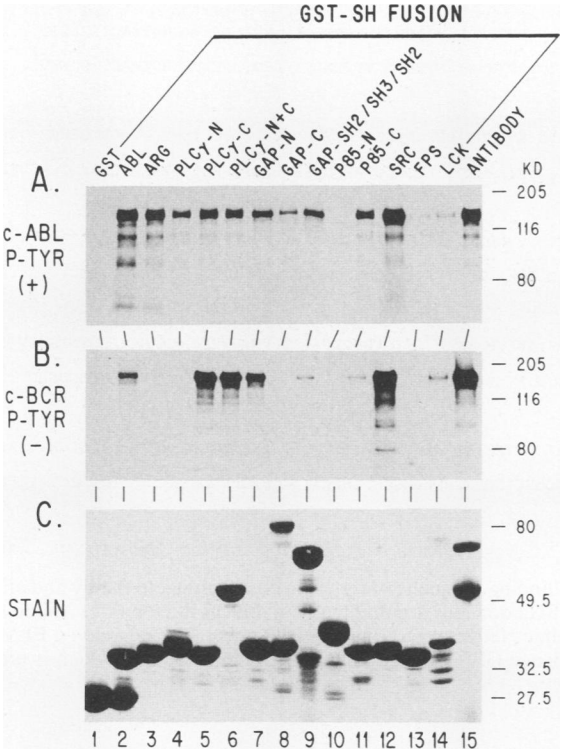

FIG. 2. Binding of tyrosine-phosphorylated c-ABL 1b and non-tyrosine-phosphorylated c-BCR by different SH2 domains. Panels A, B, and C represent three different analyses using the same set of GST fusion proteins together with an antibody control as indicated above the lanes. The antibody in panels A and C is ABL carboxy terminus-specific anti-pEX5 (17), and the antibody panel B is directed against a peptide containing amino acids 2 to 12 of BCR (33). [^{35}S]methionine-labeled c-ABL 1b (145 kDa) (A) and BCR (160 kDa) (B) produced by baculovirus expression in Sf9 cells were assayed for binding to the panel of GST-SH2 domain fusion proteins immobilized on glutathione-Sepharose beads. Binding reactions were carried out as described in Materials and Methods. (C) Approximately 15 μg of each of the GST-SH2 fusion proteins assayed for binding was loaded per lane for detection by Coomassie blue staining.

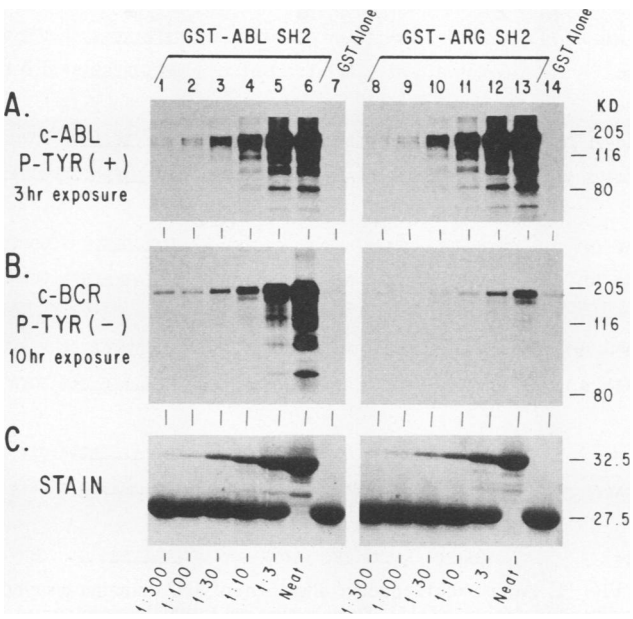

FIG. 3. Quantitative analysis of SH2 binding by tyrosine-phosphorylated c-ABL 1b and non-tyrosine-phosphorylated BCR. (C) Threefold serial dilutions of the GST-ABL SH2 fusion protein (lanes 1 to 6) and of the GST-ARG SH2 fusion protein (lanes 8 to 13) were loaded in order of increasing concentration. About 15 μg of GST-SH2 fusion protein was loaded for analysis in lanes 6 and 13 as determined by comparison of Coomassie blue-stained bands with a BSA dilution series. Dilutions of GST-SH2 fusion protein in lanes 1 to 5 and 8 to 12 were mixed with glutathione-Sepharose beads coupled to GST alone so that the total amount of protein coupled to beads in each binding reaction was maintained at a constant level. About 15 μg of GST alone was loaded in lanes 7 and 14. [^{35}S]methionine-labeled c-ABL 1b (145 kDa) (A) and BCR (160 kDa) (B) produced by baculovirus expression in Sf9 cells were assayed for binding against the two dilution series. Binding was carried out as described in Materials and Methods, except that the incubation time was increased to 6 h. Counts in bands from bound c-ABL 1b and BCR were quantitated with an AMBIS radioanalytic imaging system. Initial concentrations of c-ABL 1b and BCR in Sf9 lysate were estimated from protein immunoprecipitated with saturating amounts of antibody directed against the ABL carboxy terminus (anti-pEX5) (17) or against a peptide containing amino acids 2 to 12 of BCR (33). Counts measured in bands of Coomassie blue-visualizable amounts of immunoprecipitated protein were used to calculate counts per minute per microgram for [^{35}S]methionine-labeled c-ABL 1b and BCR.

To address which of the 10 ABL-specific amino acids might account for BCR-binding specificity, a series of composites of the ABL and ARG SH2 domains were constructed (Fig. 4A). All of the composite SH2 domains exhibited comparable binding of tyrosine-phosphorylated c-ABL 1b (Fig. 4B). Composite SH2 domains with ABL-specific amino acids in either half exhibited greater BCR-binding capability than did the ARG SH2 domain (Fig. 4C). The ABL/ARG composite bound BCR more effectively than the ARG/ABL composite did, suggesting that the N-terminal half of the SH2 domain may be more important in determining specific affinity for phosphoserine/threonine-dependent BCR. The introduction of ABL-encoded asparagine for serine or arginine for lysine at ARG-specific amino acids 9 and 35 also resulted in increased BCR-binding capability relative to the ARG SH2 domain (Fig. 4C). Thus, the BCR-binding differential between the ABL and ARG SH2 domains appears to result from the cumulative effects of several amino acid differences throughout the entire domain rather than from a single critical amino acid difference. Amino acids at the positions where the ABL and ARG SH2 domains differ are not highly conserved among the SH2 domains of other proteins (Fig. 1), and no general correlation between the amino acids at these positions and the BCR-binding capability of other SH2 domains was apparent.

The BCR-binding capability exhibited by isolated SH2 domains may be affected by additional sequences present in the normal gene product. GAP, PLCγ, and the p85 subunit of PI3 kinase each have two SH2 domains (Fig. 1). We have examined the BCR-binding capabilities of GST fusion proteins which encompass the two SH2 domains of GAP and PLCγ. One of the SH2 domains from each of these proteins bound BCR with high affinity, while the other did not (Fig. 2B). A GST fusion protein containing both SH2 domains and the SH3 domain of GAP bound relatively little BCR (Fig. 2B). This may explain why full-length, baculovirus-expressed BCR and GAP proteins did not demonstrably coim-

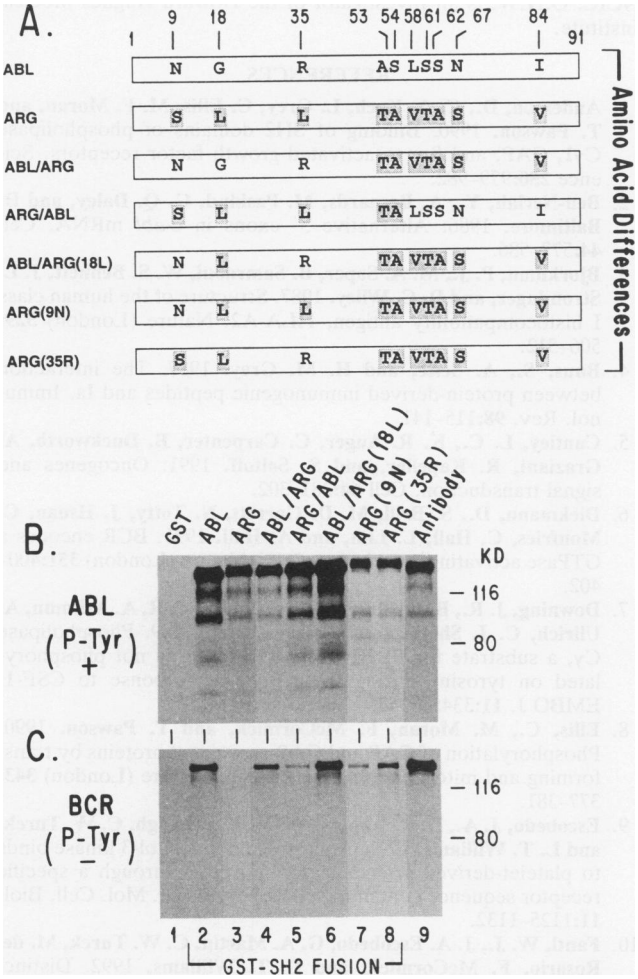

FIG. 4. Binding of tyrosine-phosphorylated c-ABL 1b and non-tyrosine-phosphorylated BCR by composite SH2 domains. Binding specificity for BCR is determined by several ABL SH2 amino acid residues. (A) The relative positions of the 10 amino acids that differ between the ABL and ARG SH2 domains are diagrammed. ARG-specific amino acids are shaded. The ABL- and ARG-specific amino acids in each of the five composite SH2 domains which were analyzed for binding are also diagrammed. (B and C) Binding of [^{35}S]methionine-labeled, baculovirus-produced c-ABL 1b (145 kDa) (B) or BCR (160 kDa) (C) to these GST-SH2 domain fusion proteins immobilized on glutathione-Sepharose beads. The order of the binding interactions tested in both panels B and C is given above the lanes. The antibody control in panel B is ABL specific, and the antibody control in panel C is BCR specific, as described in the legend to Fig. 2. Binding reactions were carried out as described in Materials and Methods.

munoprecipitate despite the relatively high BCR-binding affinity demonstrated by the isolated N-terminal SH2 domain of GAP (33). A possible explanation for this result is that *cis* linkage of the low-affinity C-terminal GAP SH2 domain impairs binding by the high-affinity N-terminal GAP SH2 domain. However, a contrasting result was obtained with the SH2 domains from PLCγ. GST fusion proteins containing both SH2 domains of PLCγ or the high-affinity C-terminal SH2 domain of PLCγ alone bound similarly high levels of BCR (Fig. 2B). This indicates that *cis* linkage of a low-affinity SH2 domain is not generally antagonistic to binding by a high-affinity SH2 domain.

DISCUSSION

We have demonstrated that only a limited subset of the SH2 domains examined bind phosphotyrosine-free BCR with relatively high affinity in vitro. It has previously been reported that phosphotyrosine-dependent binding of the PDGF receptor by either GAP or PI3 kinase can be specifically blocked by short, tyrosine-phosphorylated peptides representing sequences within the receptor (10). Similarly, binding of phosphotyrosine-free BCR by SH2 domains may be mediated through specific sites of serine or threonine phosphorylation. The SH2-binding region, which is encoded in the first exon of BCR, encompasses two serine-rich stretches, designated A and B boxes, which contain a total of 36 serines and 7 threonines (33). Specific sites of in vivo serine/threonine phosphorylation on BCR have not yet been determined. However, several discrete serine/threonine-phosphorylated fragments are generated by proteolytic digestion of a baculovirus-expressed protein segment encoded by the BCR first exon (33b). This indicates that multiple potential phosphoserine/threonine-binding sites are present in this region.

High-affinity binding of BCR by the SH2 domains examined in this study was more restricted and less avid than was high affinity binding of c-ABL 1b. This suggests that SH2-binding interactions in the cell that are phosphotyrosine independent are more selective and transient than phosphotyrosine-dependent interactions. It is not apparent from the c-ABL 1b and BCR binding data whether phosphotyrosine-dependent SH2 binding and phosphotyrosine-independent SH2 binding are both mediated through the same basic interaction or are mediated through fundamentally different types of interactions. Free phosphotyrosine at high concentrations has previously been reported to specifically compete for the SH2 binding of tyrosine phosphorylated proteins (25), although another report concludes that such competition does not occur (24). We have analyzed whether free phosphoserine or free phosphothreonine can compete specifically for ABL SH2 binding of non-tyrosine-phosphorylated BCR. However, we have not been able to demonstrate specific competition for SH2 binding with free phosphoamino acid under any circumstances, including competition for SH2 binding of tyrosine-phosphorylated c-ABL 1b with free phosphotyrosine (33a).

Of the SH2 domains examined, the ARG SH2 domain exhibits the highest degree of sequence homology with the SH2 domain of ABL, differing by only 10 of 91 amino acids. This makes its comparatively low affinity for BCR particularly striking. The BCR-binding differential between the ABL and ARG SH2 domains does not critically depend on any single amino acid difference between them. Instead, this binding differential appears to result from the cumulative effect of multiple amino acid differences between the ABL and ARG SH2 domains. Directly evaluating why the amino acid differences between the ABL and ARG SH2 domains result in the observed BCR-binding differential will require more knowledge of SH2 domain structure than is currently available. However, a model for peptide binding that is based on structural information from histocompatibility glycoproteins suggests a possible interpretation for these SH2-binding results.

Crystal structures have been determined for two class I histocompatibility glycoproteins encoded by separate alleles of the polymorphic HLA-A locus of the major histocompatibility complex (3, 11). Individual class I and class II histocompatibility glycoproteins exhibit specific binding for

a broad range of peptide sequences (4, 40). Along the solvent accessible surface of the antigen-binding cleft are six cavities, termed pockets, that are positioned to fit amino acid side chains from bound antigenic peptides (11). Amino acid differences between the two structurally characterized HLA-A alleles substantially change the charge and shape of some of the pockets while having little effect on others. It has been proposed that not all of the pockets have to be filled for binding to occur (11). This could account for the diverse range of peptide sequence-binding specificities exhibited by histocompatibility glycoproteins.

SH2 domains are functionally similar to histocompatibility glycoproteins in that they specifically bind short peptide sequences and may bind a diverse range of sequences (9, 10). The BCR-binding results obtained with the ABL and ARG SH2 domain composites are compatible with a model in which peptide-binding affinity is determined by the ability of a group of independent pockets in the SH2 domain to accommodate peptide side chains. Although the ABL and ARG SH2 domains are probably similar in overall structure, the amino acids that are different could affect enough of the requisite binding pockets to impair the ability of the ARG SH2 domain to effectively bind to the site in BCR which is bound by the SH2 domain of ABL. The effect on BCR-binding capability of substituting ABL-specific amino acids at different positions in the ARG SH2 domain would be cumulative if different pockets were affected.

The potential for specific interactions between BCR and SH2-containing proteins is particularly intriguing in light of functional characteristics recently ascribed to BCR. These include rac-GAP activity (6) and serine/threonine kinase activity (23). BCR has also been reported to have homology with the catalytic region of the GTP-GDP exchange protein dbl (12). Interactions mediated through the BCR SH2-binding domain may be important for transformation by the *BCR/ABL* chimeric oncogene as well. This possibility is supported by the observation that removal of sequences spanning the entire SH2-binding region of P185 abrogates its transforming ability (33).

If phosphotyrosine-independent SH2-binding interactions are responsible for the formation of specific protein-protein complexes, then SH2 domains may exhibit selective binding affinities for different substrates. When additional serine/threonine-phosphorylated, SH2-binding substrates are identified, it will be interesting to determine how binding of these proteins compares to binding of BCR by different SH2 domains. p190 is a potential candidate which is phosphorylated predominantly on serine in vivo and which binds to GAP and affects its activity (8, 30). It is also possible that some of the substrates for tyrosine phosphorylation by cytoplasmic tyrosine kinases, such as the p62 GAP-binding protein (8), initially interact with SH2 domains through a phosphotyrosine-independent interaction. Studies addressing these particular issues should be facilitated by the recent identification of cDNA clones encoding both p190 (37) and p62 (43).

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